AR201-14084B

Substance Group: Group 27

Summary Prepared by: Petroleum Additives Panel
Health & Environmental Research Task Group

Date of last update: November 26, 2002

Contact:

Sarah Loftus McLallen
American Chemistry Council
1300 Wilson Boulevard
Arlington, VA 22209
1-703-741-5607 (phone)
1-703-741-6091 (fax)

Sarah_McLallen@americanchemistry.com

1.0 Biodegradation

Robust Summary 27-BioDeg-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 days
Test apparatus	Six glass 4-liter Erlenmeyer flasks containing two liters of modified biochemical oxygen demand water (BOD).
Inoculum	Activated sewage sludge from a domestic wastewater treatment plant, prepared per test guideline. Inoculum was not acclimated. Twenty milliliters of inoculum added to all test flasks.
Replicates:	Duplicates for test substance and blank controls, single flasks for the reference substance and toxicity control.
Temperature of incubation:	21.6-23.4 °C
Dosing procedure:	Neat test chemical was gravimetrically determined on glass cover slips, which were then added to culture medium in test vessels.
Study initiation:	Test flasks provided with CO_2 free air and placed on a rotary shaker and mixed at 110rpm for the study duration.
Sampling:	Days 2, 4, 7, 10, 14, 17, 21, 24, 29 (after acidification on day 28)
Concentration of test substance:	10 mg C/L weighed directly onto tared glass slides and placed into each test substance flask.
Controls:	Toxicity, blank and positive controls used per guideline. Positive control was benzoic acid (Na salt) added to the control vessel at a loading of 10 mg C/L. Both test substance and reference material were added to the toxicity control flask to obtain a maximum concentration of 20 mg C/L
Analytical method:	Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution, using 0.05N HCl.
Study termination:	The pH of the content of each test flask was determined. The flasks were then acidified with 1 ml of concentrated sulfuric acid to drive off inorganic carbonate. A sample was centrifuged and a subsample of the concentrate was submitted for soluble organic carbon (SOC) analysis.
Method of calculating biodegradation values:	Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	The test substance was not considered readily biodegradable under the criteria that requires 60% biodegradation within 28 days, achieved within 10 days of reaching 10% biodegradation. The CO_2 production from the reference chemical exceeded the 60% of theoretical necessary to consider the test valid. The % TCO_2 for the toxicity control was > 25 as of day 12 therefore the test substance was not considered to be inhibitory at the concentration tested.
Degradation % After Time	Test substance: 2.3-5.0 % TCO_2 in 28days Positive control substance: 88.1% in 28 days
Conclusions	The test substance was not readily biodegradable (2.3-5.0% TCO_2 in 29 days).
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 11/13/2002

2. 0 Acute Toxicity to Fish

Robust Summary 27-FISH-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rainbow Trout (*Oncorhynchus mykiss*)
Fish Number	20/concentration (10/exposure chamber, duplicate chambers/concentration; 0.83 grams of fish/liter)
Fish Size	Average length 50.3 mm; Average weight 1.25 g
Analytical Monitoring	Yes (Total Organic Carbon determinations)
Nominal Test Substance Concentration Levels	0, 130, 220, 370, 600 and 1000 mg/l
Test Concentration Preparation	Test solutions were prepared separately for each replicate test concentration by adding an appropriate aliquot (by weight) of test material to 35 liters of dilution water in glass vessels. The solutions were stirred vigorously for approximately 20 hours. Following a settling period of 4 hours the water accommodated fraction (WAF) of each replicate test concentration was siphoned into each replicate test vessel.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. At 24, 48 and 72 hours of exposure the test fish were carefully transferred into aquaria containing fresh test solutions.
Vehicle	None
Statistical Analysis	None required based on the results.
Dose Rangefinding Study	Yes
Test Chambers	20-liter glass aquaria containing 15 liters of test solution
Diluent Water	Deionized water
Diluent Water Chemistry	Hardness 40-48 mg/l as $CaCO_3$ Alkalinity 39 mg/l as $CaCO_3$ Conductivity 130 umhos/cm pH 7.5-7.8
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 7-9.8 mg/L pH: 6.5-8.1 Conductivity: 120-160 umhos/cm
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	11-13 ^{O}C during 14 day holding period 11-12 ^{O}C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 2, 24, 48, 72, and 96 hours after initiation of test material exposure.

Results	Analysis of freshly prepared and 24 hour control and test solutions for total organic carbon resulted in the following results: Nominal WAF Concentration (mg/L) / TOC (mg/L) 0 hr / TOC (mg/L) 24 hr 0 (control) 2.1-2.2 3.2-4.2 130 2.8 2.7-3.3 1000 4.8-5.0 6.2-6.5 Throughout the test period a film of dissolved test material was observed on the surface of test solutions at a concentrations of 220 mg/L WAF and higher. WAFs at a concentration of 370 mg/L and higher were slightly cloudy for at least a portion of the 96 hour test period. Insoluble test material was not noted in any other test solution. Fish in the negative control group were normal throughout the study. No mortality was observed at 130, 220 or 600 mg/L. At 370 mg/L 10% mortality was observed at 96 hours. At 1000 mg/L 15% and 25% mortality were observed at 72 and 96 hours respectively. At 48 and 72 hours some fish at 1000 mg/L appeared lethargic and exhibited a loss of equilibrium. The mortality and other effects observed at higher loading rates may have been due to physical effects of undissolved test material (e.g. fouling of gills). The 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (WAF). The 96 hour no observed effect level was 600 mg/L.
Conclusions	Under the conditions of this study the 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (WAF). The 96 hour no observed effect level was 600 mg/L.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/13/2002

3.0 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 27-DAPH-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Purity not provided
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	*Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at 48 hours post initiation of exposure.
Exposure Period (unit)	48 hours
Statistical methods	Based on 100% survival no statistical analysis was required.
Remarks field for test conditions (fill as applicable)	Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1.5 L) in a glass vessel and stirred for 20 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. Following the mixing period, the test solutions were allowed to stand for 4 hours before the water phase was gently siphoned from the mixing vessel into corresponding replicate test vessels (1 liter/vessel). The toxicity test was conducted in 300 ml glass beakers that contained 250 ml of test solution. Thirty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate) within 30 minutes of test solution preparation. At 24 hours the test solutions were replaced with newly prepared WAF and all surviving daphnids were carefully transferred into the corresponding test vessel. Daphnids were not fed during exposure. Control test chambers/daphnids were handled in an identical fashion. Light cycles were maintained at 16-hour light per day with an intensity of 7 uEin/m^2sec. Test solutions were maintained at 20 $\pm$ 1 degree C. Dilution water was filtered well water adjusted to the appropriate hardness of 160-180 mg/L as $CaCO_3$.
Test Concentrations	0 and 1000 mg/L WAF
RangeFinding Study	Yes; concentration range of 10 to 1000 mg/L WAF
Results	24 and 48-h EC_{50} >1,000 mg/L (WAF). 48 hour NOEC is 1000 mg/L.
Remarks	Water chemistry: Dissolved oxygen: 7.7-8.5 mg/L; pH: 7.5 - 8.0; conductivity: 560 – 600 umhos/cm. Total Organic Carbon measurements were 6.4 mg/L and 2.2mg/L in the 1000 mg/L test concentration solution and in the control solution at

	48 hours. Analysis of 0 hour test solutions resulted in measurements of 1.2mg/L and 6.4mg/L in the control and 1000 mg/L test concentration solution. No insoluble test material was noted during the study. Following 48 hours of exposure, no dead or immobilized organisms were observed at the treatment level (1000 mg/L). . The 48-hour EC50 was determined to be greater than 1000 mg/L, the only concentration tested. The no observed effect concentration was established as 1000 mg/L.
Conclusions	The 24 and 48-h EC_{50} >1,000 mg/L (WAF). The no observed effect concentration was established as 1000 mg/L.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/28/2002

4.0 Acute Toxicity to Aquatic Plants (e.g. algae)

Robust Summary 27-ALG-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1993), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) and final (96-h) control and test solutions followed EPA Method 415.1 (unfiltered).
Statistical methods	The 24, 48, 72 and 96 hour effective concentrations (EC10, EC50, EC90) could not be calculated using standard statistical techniques because cell growth at the single tested concentration equaled 90 to 100 % of the cell growth of the controls throughout the test. The 96 hour NOEC was determined using TOXSTAT 3.3, which calculated a t-test, comparing the growth of algae of the controls to the growth of algae in the treated group.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from the University of Texas at Austin alga collection. Test System: The WAF was prepared only at the beginning of the test. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 20 hours. Stirring accomplished using a magnetic stirrer. Mixing speed was adjusted such that a vortex formed approximately 25% of the distance to the bottom. Following the mixing period, the test solution was allowed to stand for 4 hour before the water phase was removed. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were covered to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts, the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of

	cells to test containers or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of approximately 400 foot-candles. Test temperature: 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.4 at 0-hour and 9.6 - 10.6 after 96 hours. Test Levels: Control and 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC levels were non-detect (<1) – 2.1 mg/L in control and 5.8 – 6.7 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	96-h EC_{50} >1,000 mg/L; The 96-hr NOEC = 1,000 mg/L.
Remarks	Test Findings: At 96-hours biomass measurement in the treated group was 95% of the control at 1,000mg/L (WAF). Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at a loading rate of 1,000 mg/L. 96-h EC_{50} >1,000 mg/L; The 96-hr NOEC = 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Confidential business information.
Other	Updated: 11/13/2002

5.0 Acute Oral Toxicity

Robust Summary 27-AcuteOral-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Sprague-Dawley
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	5.56 ml/kg
Control group	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats. Clinical observations were conducted at 1, 2.5, and 4 hours after test material administration and daily thereafter for 14 days. The animals were observed for mortality twice daily. Individual body weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. All animals exhibited body weight gains during the study. All animals appeared normal throughout the study. There were no significant necropsy findings evident in the surviving animals.
Conclusions	The test article, when administered to 5 male and 5 female rats had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/27/01

Robust Summary 27-AcuteOral-2

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/ Wistar strain
Sex	Male
No. of animals/dose	10
Vehicle	Mazola Corn Oil (50% mixture)
Route of administration	Oral (intragastric)
Dose level	5, 7.12, 10.14 and 14.43 g/kg
Dose volume	Not provided
Control group included	No
Remarks field for test conditions	A single dose of the test material was administered intragastrically to 10 fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes 3-4 hours after dosing and daily for 14 days. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 >14.9 g/kg (males)
Remarks	All of the animals survived the study. At 14.43 g/kg diarrhea, chromorhinorrhea and oily bodies were noted in 5 or more animals. Isolated instances of ptosis, chromodacryorrhea and piloerection were also noted. At 10.14 g/kg diarrhea, chromorhinorrhea, piloerection, ptosis and lethargy were noted in 5 or more animals. Isolated instances of ataxia and chromodacryorrhea were also noted. At 7.12 g/kg isolated instances of diarrhea, piloerection, lethargy and respiratory noise were noted. At 5.0 g/kg isolated instances of diarrhea and chromorhinorrhea were noted. All animals gained body weight during the study. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered to male Wistar rats, had an acute oral LD50 of >14.9 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 1/21/02

6.0 Acute Dermal Toxicity

Robust Summary 27-Acute Dermal-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg (0.014 g/cm^2)
Application area	Approximately 36 cm^2
Control group included	No
Remarks field for test conditions	Prior to the initiation of dosing the back and flanks of each animal were clipped of hair to expose 20% of the total body surface. Animals were reclipped as needed. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female rats. The test material was kept in contact with the skin for a period of 24 consecutive hours, on approximately 20% of the total body surface under a semi-occlusive bandage that was loosely over wrapped with a sheet of perforated plastic film. At the end of the 24-hour exposure period, the application site was wiped clean of residual test material with mineral oil, followed by liquid Ivory soap mixed with warm tap water, rinsed with tap water, and dried with a paper towel. The animals were observed for abnormal clinical signs at 1, 2.5, and 4 hours after dosing and daily for the 14-day study period. Dermal examinations were performed 30 minutes post test material removal and on days 3, 7, 10 and 14 according to the Draize method. Individual body weights were recorded on day 1, prior to dosing, and on days 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical observations were unremarkable. All animals exhibited body weight gains during the study. A slight erythema and edema reaction was observed in one male (days 1 and 3). Slight erythema was observed in one female (day 1). These findings cleared by day 7 in the male and by day 3 in the female. There were no macroscopic findings associated with treatment.

Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague-Dawley rats, had an acute dermal LD50 of greater than 2.0 g/kg. Evidence of slight dermal irritation was observed in two animals during the first week of study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary 27-AcuteDermal-2

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rabbits/New Zealand White
Sex	Male
No. of animals/group	4
Vehicle	None
Route of administration	Dermal
Dose level	20 g/kg
Dose volume	Not provided.
Control group included	Yes
Remarks field for test conditions	This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of 2 of 4 treated animals was abraded prior to dosing. In addition the guideline calls for the evaluation of males and females using at least one dose level. This study was conducted using males only. These deviations were not considered sufficient to change the outcome of the study. Immediately prior to topical application of the test material, the abdominal hair of each animal was closely clipped. The skin of two of the four treated animals was abraded prior to test material administration. A single dose of 20 g/kg of the undiluted test material was administered dermally to four male animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment. Irritation was scored (Draize) on day 1 post treatment. All animals were euthanized at the conclusion of the observation period. Gross necropsies were not performed.
Results	LD50 > 20.0 g/kg (males)
Remarks	All animals survived the duration of the study. Lethargy, diarrhea, bloated abdomen and difficulty walking due to soreness of the exposure area were noted in 2 or more animals. Isolated instances of mucus in the stool were also noted. Slight to well defined erythema and very slight-to-slight edema were noted in the treated skin of all animals at 24 hours post dosing. Body weight data was unremarkable.

Conclusions	The test article, when administered dermally as received to 4 male New Zealand white rabbits had an acute dermal LD50 of greater than 20.0 g/kg (males)
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 1/21/02

7. 0 Gene Mutation Assays

Robust Summary 27-GenTox-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate Confirmatory assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F127 (25% w/w in ethanol)
Vehicle Control	Pluronic F127 (25% w/w in ethanol)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 25.0 ug/plate WP2*uvr*A –S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2*uvr*A and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a non-cytotoxic dose level of test article (5000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.

Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. Plates that were not evaluated immediately were held at 5^{o}C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. The number of revertant colonies/plate for the vehicle controls and all plates containing test article were counted manually. The number of revertant colonies/plate for the positive controls were counted by automated colony counter.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In the dose rangefinding study no cytotoxicity was observed with tester strain TA100 or WP2*uvr*A at dose levels up to 10,000 ug/plate with or without metabolic activation. Test article precipitate was observed on plates at 667 ug/plate and above with tester strain TA100 with activation and at 1000 ug/plate and above without activation. With WP2*uvr*A without metabolic activation precipitate was observed at 667 ug/plate and above. With activation, with WP2*uvr*A, precipitate was observed at 333 ug/plate and above. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected. The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (5000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study. In the initial assay all data were acceptable and a dose responsive increase in the mean number of revertants/plate was observed with tester strain TA100 only in the absence of activation. In addition non-dose responsive increases were observed with TA98 and WP2*uvr*A with activation. These results were unexpected in light of the range find work and the S-9 optimization study. For these reasons the confirmatory assays were conducted using the same dose levels. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on these results all tester strains were retested for confirmation again. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed. Based on these results the test material was considered not mutagenic. The initial positive results seen in three tester strains were considered spurious.

	No cytotoxicity was observed up to 10,000 ug/plate with the *Salmonella* tester strains with and without activation and with WP2*uvr*A with and without activation. Test material participate was observed on plates at ≥250 ug/plate. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/01

Robust Summary 27-GeneTox-2 --- MORE THAN ONE STUDY AVAILABLE!!

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	77% Active Ingredient
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	N
Year (Study Performed)	1979
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.33, 1.0, 3.33, 10.0 and 33.3 ul/plate
Metabolic Activation	With and without (0.5 ml of S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 Aflatoxin B1 1.0 ug/plate TA98 -S9 2-nitroflourene 0.5 ug/plate TA100 +S9 Aflatoxin B1 1.0 ug/plate TA100 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 9-aminoacridine 100 ug/plate TA1538 +S9 2-aminofluorene 2.0 ug/plate TA1538 -S9 2-nitroflourene 5.0 ug/plate
Vehicle Control	Ethanol 100 ul/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point. Linear regression analysis was used to compute the best-fit line of dose response.
Dose Rangefinding Study	No
S9 Optimization Study	Yes
Remarks field for test conditions	This study was conducted in 1979, prior to the adoption of OECD Test Guideline 471. In addition to the tester strains used during this study, the OECD Guideline suggests the inclusion of tester strains *E.coli* WP2 uvrA, or WP2 uvrA (pKM101) or *Salmonella typhimurium* TA102. This study included the use of tester strain TA1538. OECD 471 does not incorporate this strain. These deviations from the test guideline are not considered major study deficiencies. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle control, and a positive control. Three plates/dose group/strain/treatment set were evaluated. Test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, and S9 mix (if needed). This was overlaid onto the

	surface of supplemented Noble's agar in a screw-capped tube. Tubes were mixed and poured over a base plate of Spizzizen's minimal medium. Plates were incubated for 48 hours at 37°C.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In this mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. For each strain, the numbers of revertant colonies in negative control plates were within acceptable limits as defined by historical control data for spontaneous revertants. Sterility controls were negative.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/13/01